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Hamajima et al.

[11] **Patent Number: 5,776,758**

[45] **Date of Patent: Jul. 7, 1998**

[54] **CYSTEINE PROTEASE DERIVED FROM PARASITIC HELMINTHS**

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[21] Appl. No.: **451,409**

[22] Filed: **May 26, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 246,917, May 20, 1994, abandoned, which is a continuation of Ser. No. 920,092, Jun. 24, 1992, abandoned.

[30] **Foreign Application Priority Data**

Jul. 25, 1991 [JP] Japan 3-208546
Feb. 12, 1992 [JP] Japan 4-057189

[51] **Int. Cl.**^{6} **C12N 9/50**
[52] **U.S. Cl.** **435/219**; 435/212
[58] **Field of Search** 424/94.63, 94.65; 435/219; 514/2, 12

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Assistant Examiner—Enrique Longton
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[57] **ABSTRACT**

The immunosuppressive drug of the present invention can suppress both delayed-type hypersensitivity and antibody production against specific antigens and graft tissues and induce immunological tolerance to them by several administrations, instead of long continuous administration.

Cysteine protease, a secretory protein accumulated in the tissue of parasitic helminths, is extracted and purified. The cysteine protease is administered to a mammal and then tissue is implanted to the mammal. The immune response of the mammal against the tissue implant is suppressed even one year later.

13 Claims, 6 Drawing Sheets

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                                                          22  25
a. Cysteine protease               APERIDWRAKGAVT-AVENQGSCGSCWAFSTAGN
b. PAPAYA PROTEINASE OMEGA         LPENVDWRKKGAVT-PVRHQGSCGSCWAFSAVAT
c. PAPAIN PRECURSOR                IPEYVDWRQKGAVT-PVKNQGSCGSCWAFSAVVT
d. CHYMOPAPAIN                     YPQSIDWRAKGAVT-PVKNQGACGSCWAFSTIAT
e. CATHEPSIN L                     APRSVDWREKGYVT-PVKDQGQCGSCWAFSTTGA
f. CATHEPSIN H PRECURSOR           YPPSVDWRKKGNFVSPVKNQGACGSCWTFSTTGA

                         56      63
a. VEGQWFIKTGQLVSLSKQQLVDCDRVA--QGCNGGWPASSYLEIMYM
b. VEGINKIRTGKLVELSEQELVDCERRS--HGCKGGYPPYA-LEYVAK
c. IEGIIKIRTGNLNEYSEQELLDCDRRS--YGCNGGYP-WSALQLVAQ
d. VEGINKIVTGNLLELSEQELVDCDKHS--YGCKGGYQTTS-LQYVAN
e. LEGQHFRTKGKLVSLSEQNLVDCSRPEGNQGCNGGLMDQAFQYVQDN
f. LESAIAIATGKMLSLAEQQLVDCAQDFNNYGCQGGLPSQAFEYILYN

                  96
a. GGLESESDYPYVGVEQT-C-ALNKEKLVAKIDDSIVLGPEEEDHAAY
b. NGIHLRSKYPYKAKQGT-CRAKQVGGPIVKTSGVGRVQPNNEGNLLN
c. YGIHYRNTYPYEGVQRY-CRSREKGPYAAKTDGVRQVQPYNEGALLY
d. NGVHTSKVYPYQAKQYK-CRATDKPGPKVKITGYKRVPSNCETSFLG
e. GGIDSEESYPYTAKDDEDCRYKAEYNAANDTGFVDIPQGHERALMKA
f. KGIMGEDTYPYQGKDGY-CKFQPGKAIGFVKDVANITIYDEEAMVEA
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FIG. 1A

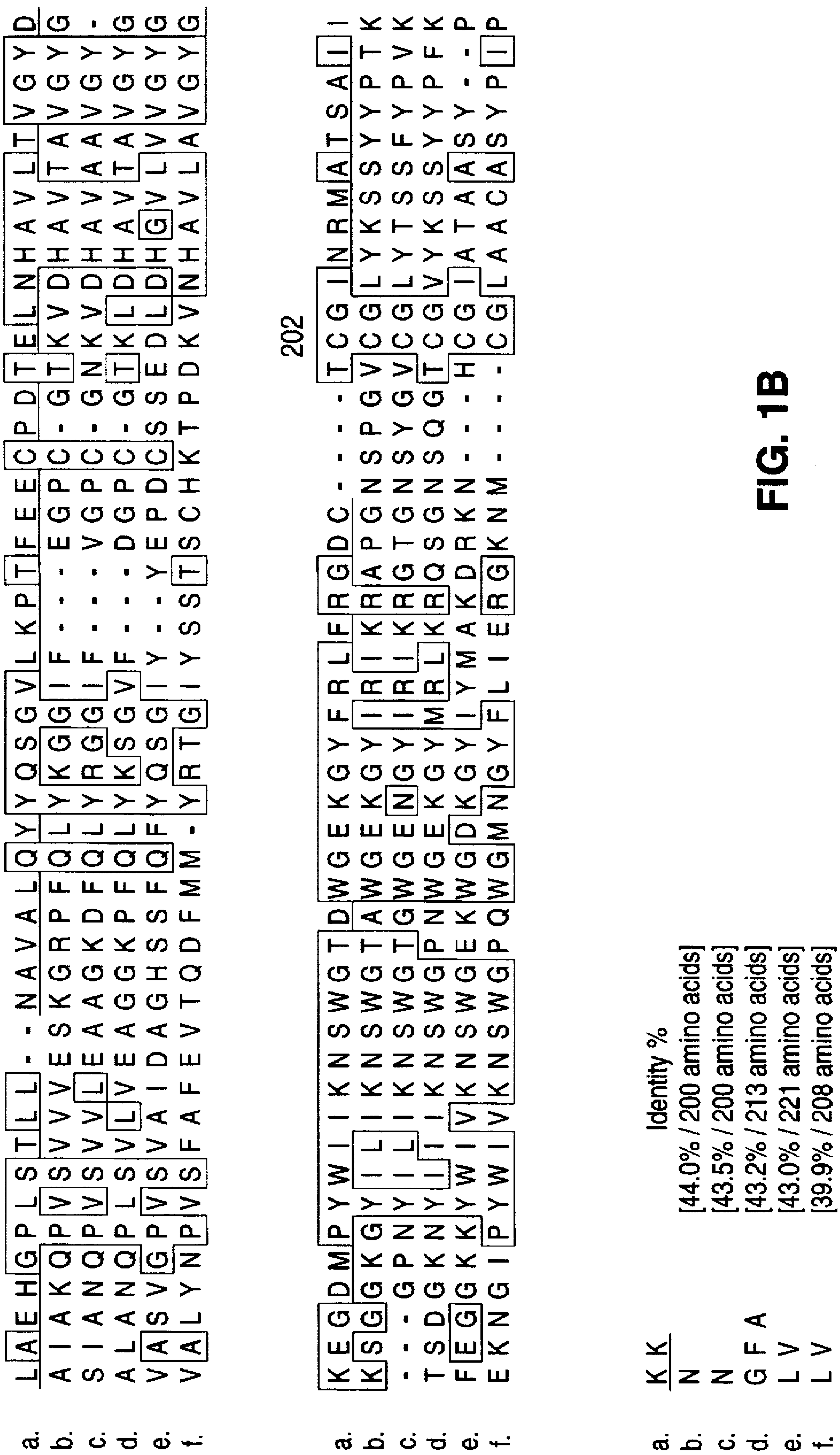
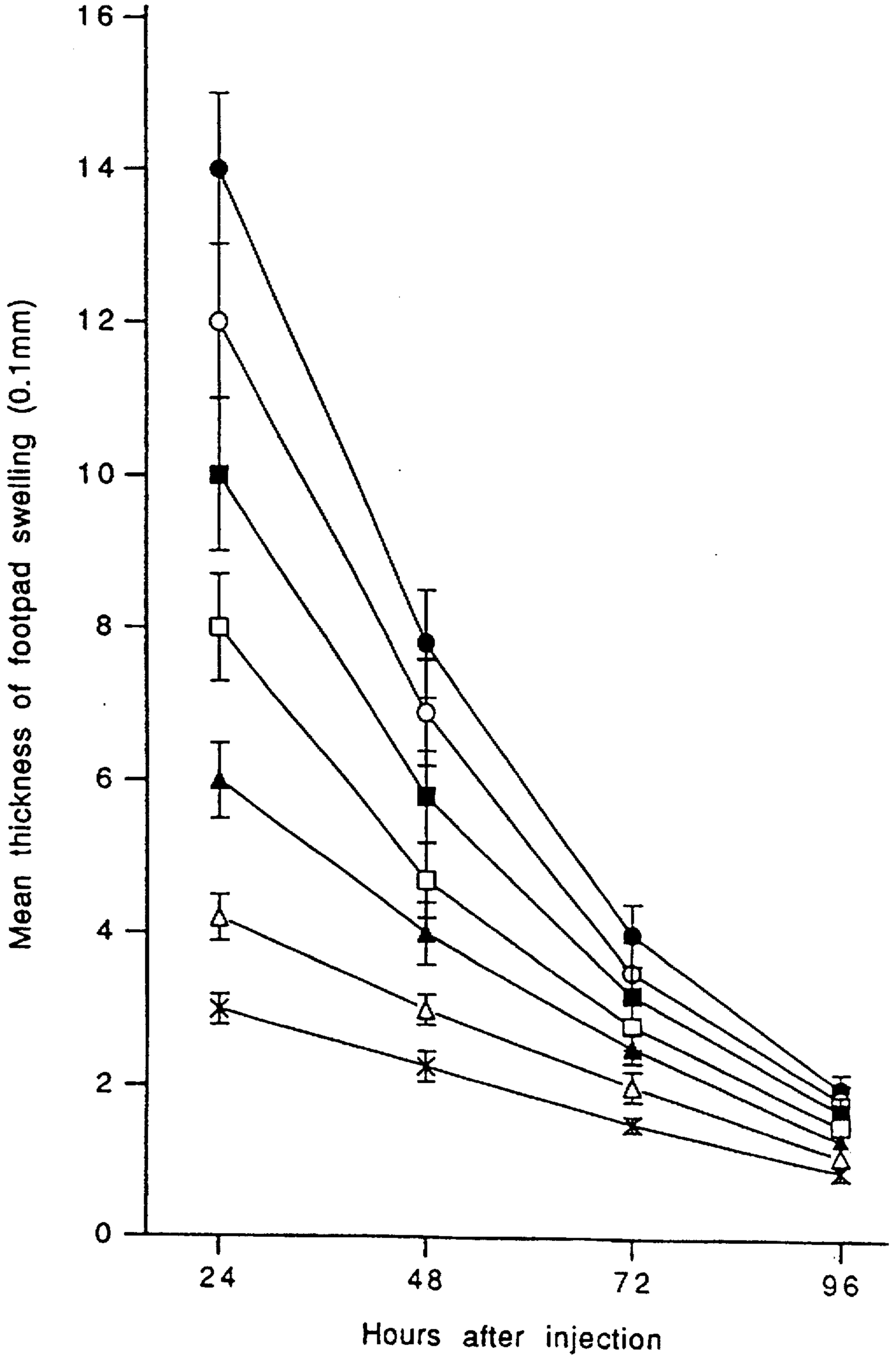

154
161
202
Identity %
[44.0% / 200 amino acids]
[43.5% / 200 amino acids]
[43.2% / 213 amino acids]
[43.0% / 221 amino acids]
[39.9% / 208 amino acids]

FIG. 1B

FIG. 2


Mean thickness of footpad swelling (0.1mm)
0
2
4
6
8
10
12
14
16
24
48
72
96
Hours after injection

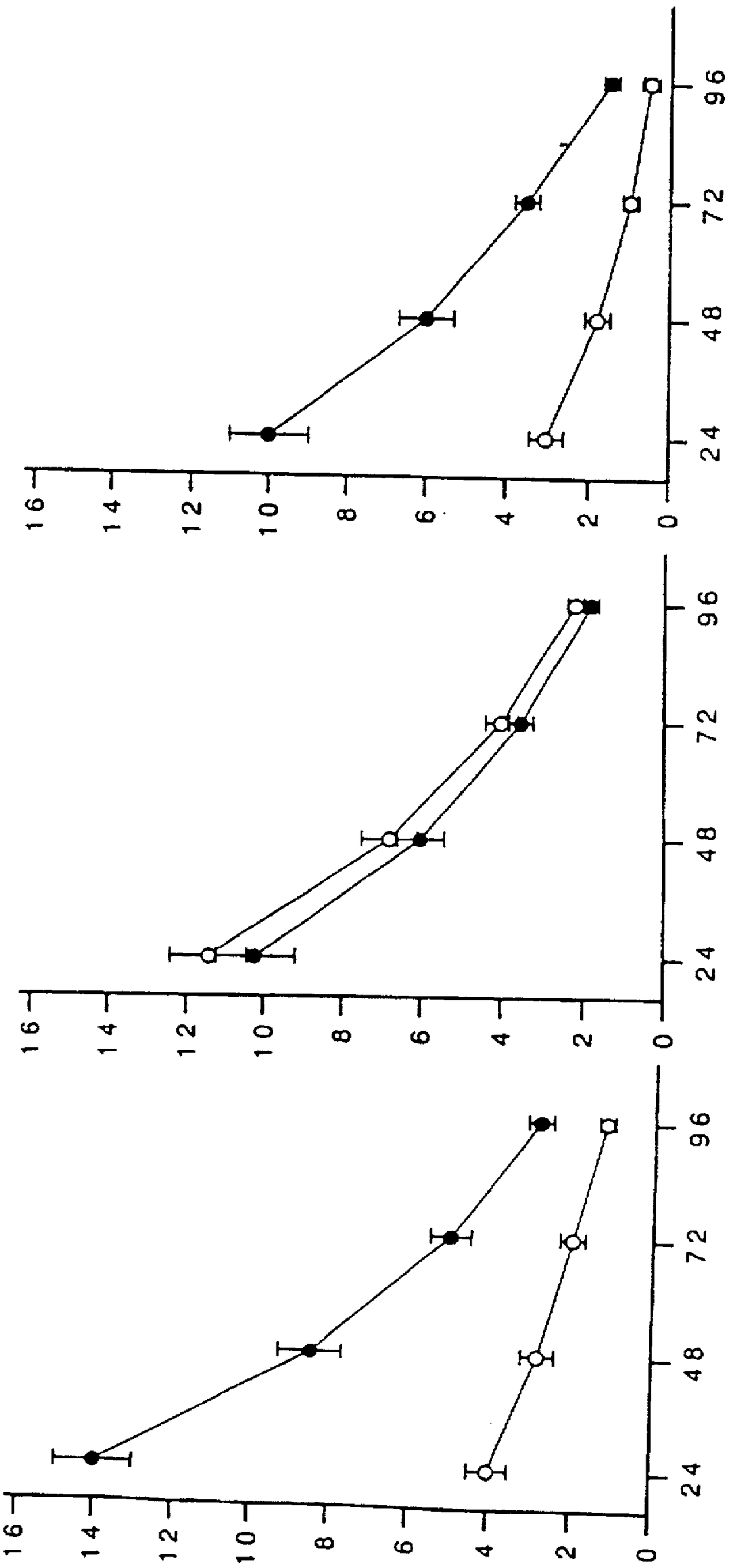


FIG. 3a

FIG. 3b

FIG. 3c

FIG. 4c

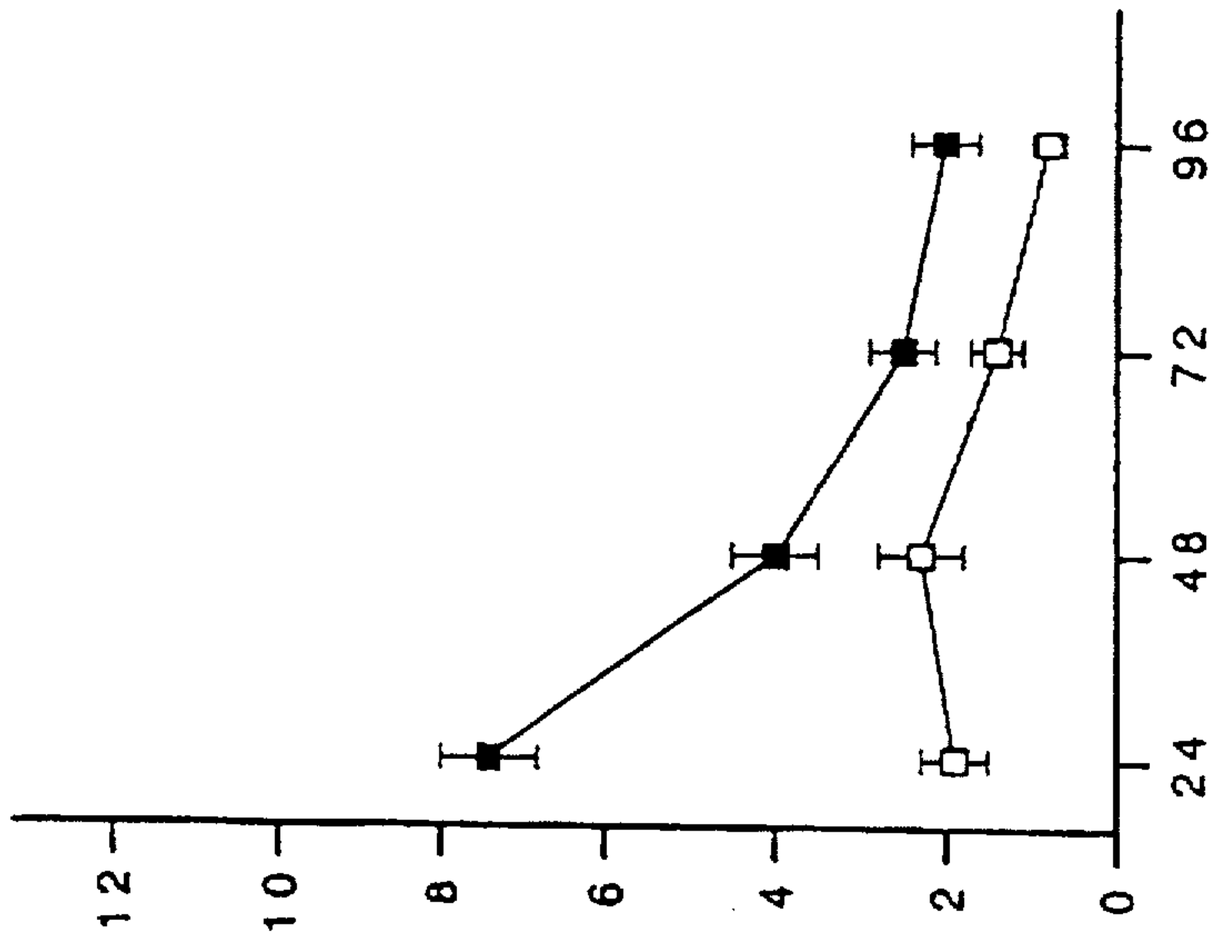


FIG. 4b

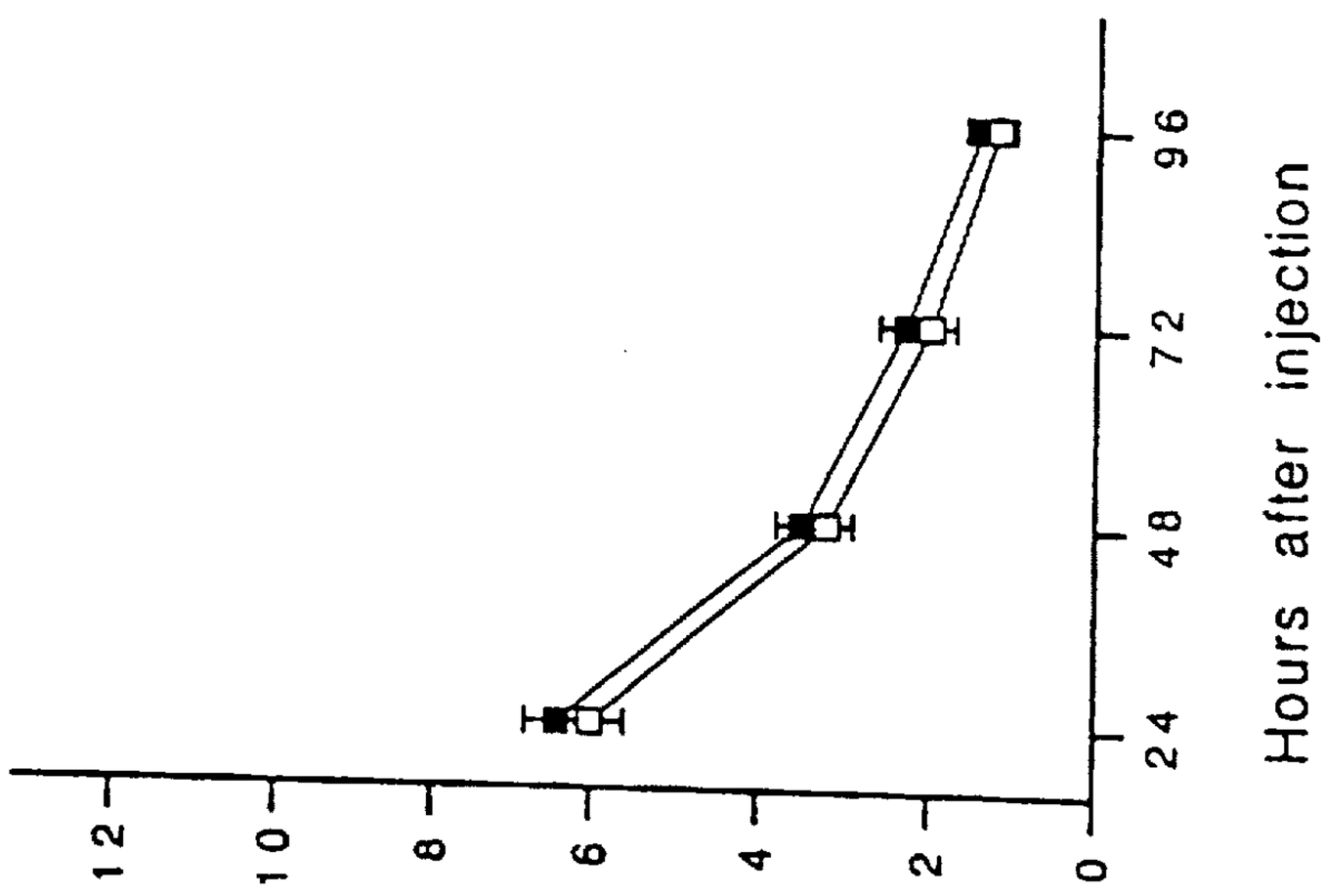


FIG. 4a

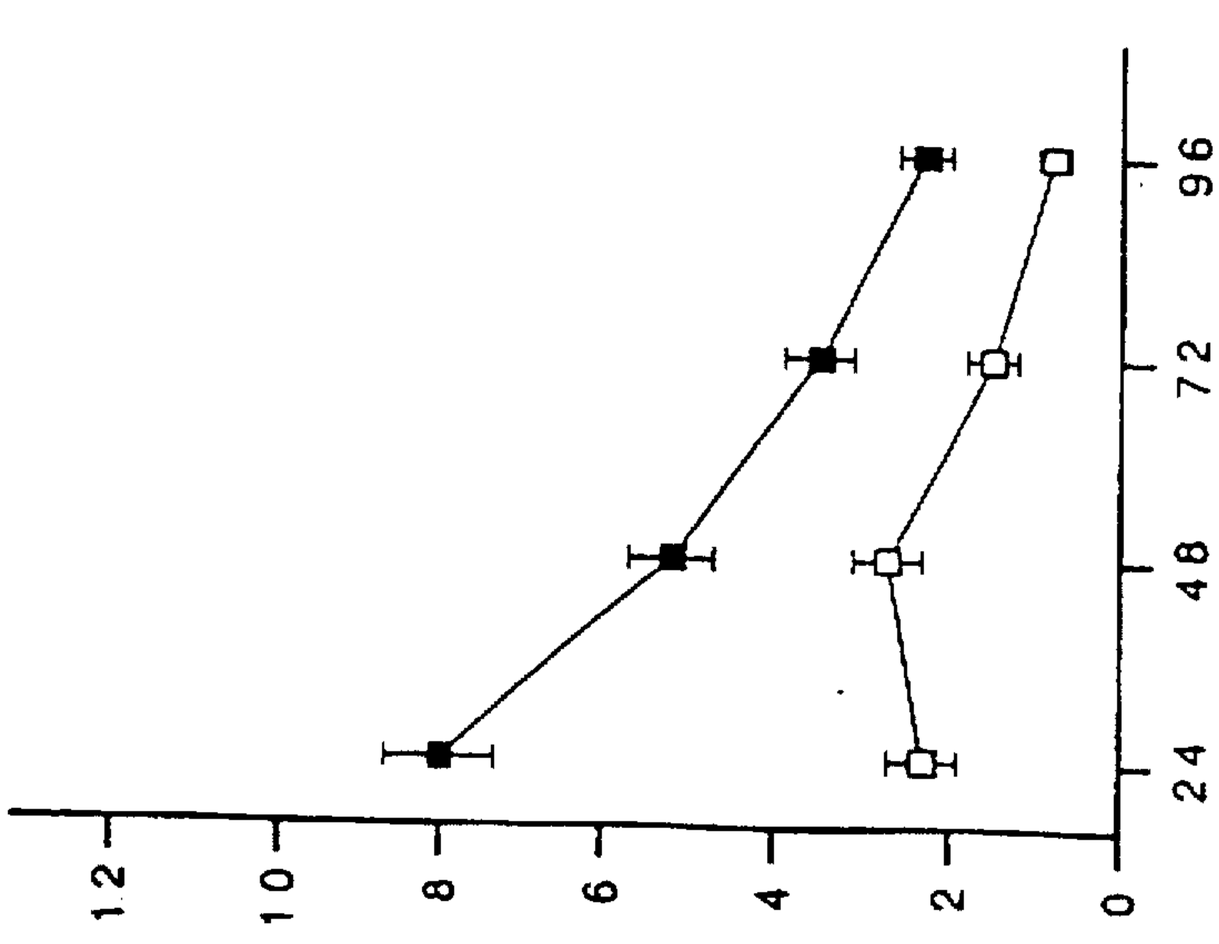
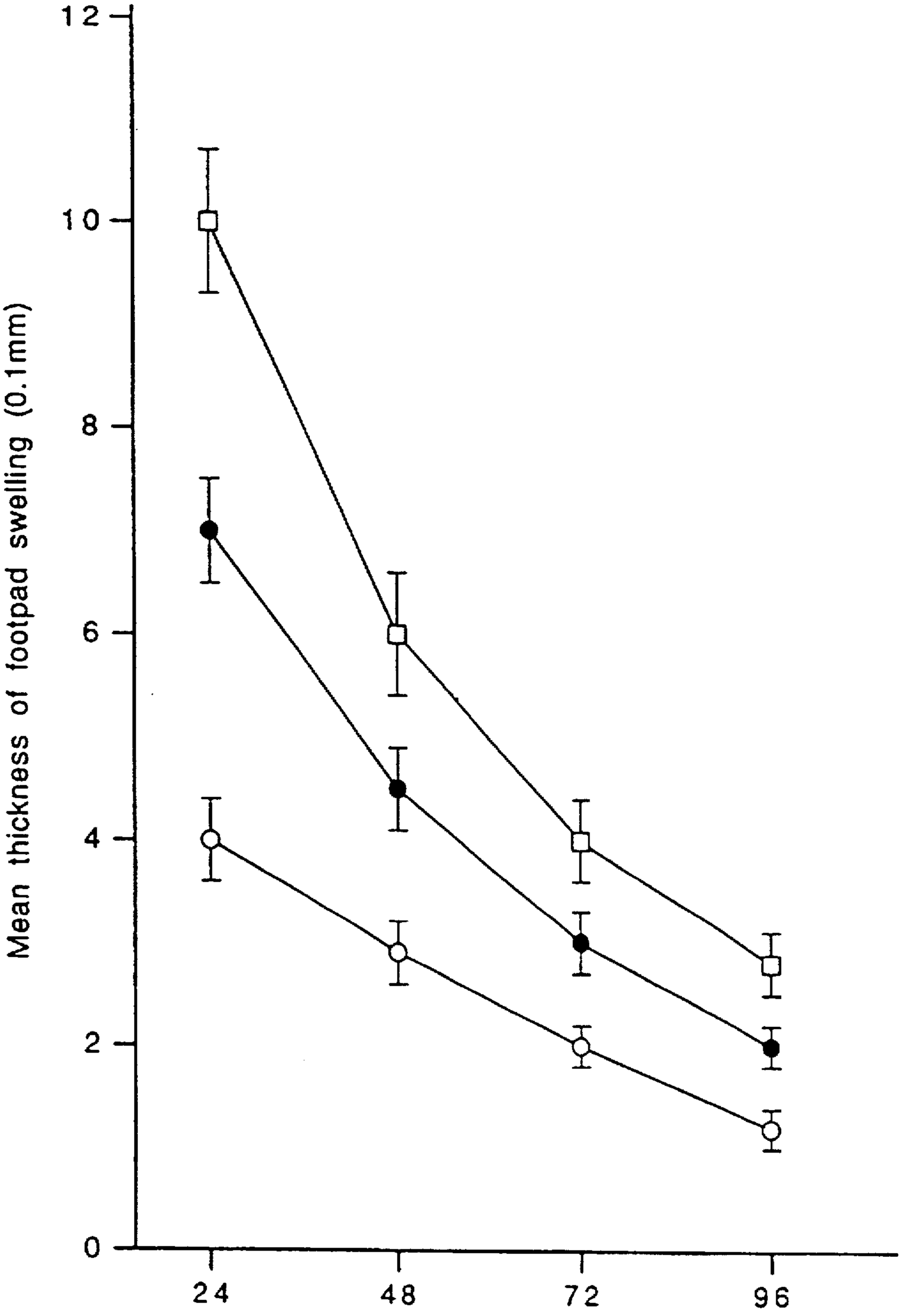


FIG. 5


Mean thickness of footpad swelling (0.1mm)
12
10
8
6
4
2
0
24
48
72
96
Hours after injection

CYSTEINE PROTEASE DERIVED FROM PARASITIC HELMINTHS

This is a continuation of application Ser. No. 08/246,917 filed on May 20, 1994 which is a File Wrapper Continuation application of Ser. No. 07/920,092 filed on Jun. 24, 1992, both now abandoned.

FIELD OF THE INVENTION

The present invention relates to immunosuppressive drugs used for suppression of graft rejection in organ transplantation or suppression of autoimmune diseases.

BACKGROUND OF THE INVENTION

Currently, azathiopline, cyclosporin A, FK-506, and 15-deoxysperguarine are known as immunosuppressive drugs used for a therapeutic purpose, and have potent suppression effect.

However, the conventional immunosuppressive drugs do not induce immunological tolerance and are therefore required that the drug be continuously administered for a long period of time. Side effects such as cancer, nephrotoxicity, angiitis, hepatotoxicity and disorders in the digestive tract have resulted from the continuous administration of these doses.

The present inventors have studied agents of human parasites, especially parasitic helminths, which protect them from the immune response of human hosts, and have found that a cysteine protease, a secretory protein accumulated in the tissue of parasitic helminths, suppresses the cell-mediated and humoral immunities of mammalian hosts and induces the hosts to acquire immunological tolerance to the parasite.

SUMMARY OF THE INVENTION

The immunosuppressive drug of the present invention can suppress both delayed-type hypersensitivity and antibody production against specific antigens and graft tissues, and induce immunological tolerance by several administrations. Conventional immunosuppressive drugs are required that they be administered for a long period of time, and cause an adverse effect which is a drawback of the drugs. In contrast, it is not required that the immunosuppressive drug of the present invention it be administered for a long period of time. Therefore, the immunosuppressive drug of the invention overcomes the drawback of conventional immunosuppressive drugs.

DESCRIPTION OF THE FIGURES

FIGS. **1A** and **1B** show a comparison of the deduced amino acid sequences of a cysteine protease from *Paragonimus westermani* metacercariae and other similar cysteine proteases. Homologous areas are boxed and heterologous amino acids are underlined.

FIG. **2** is a graph showing a footpad response (swelling of footpad) of C_{57}BL/6 female mice. The female mice were intraperitoneally injected with sheep red blood cells (SRBC) at various times after intraperitoneal injection of the present cysteine protease. The intervals were 0(X), 1(Δ), 2(▲), 3 and 4(□), 5 and 6(■), 7 and 8 days (○) and untreated control (●). Bars represent standard errors.

FIGS. **3A**, **3B** and **3C** are graphs showing a footpad response of C_{57}BL/6 female mice to sheep red blood cells (SRBC) and adult *Paragonimus westermani* antigens. The mice were immunized with SRBC one day after an injection of the present cysteine protease. Group treated with the protease on day-1 (○) and control group (●). Bars represent standard errors. (a) The initial SRBC injection one day after the protease treatment. (b) Adult *P. westermani* antigen injection six months after the initial SRBC immunization. (c) SRBC injection one year after the initial SRBC immunization.

FIGS. **4A**, **4B** and **4C** are graphs showing a footpad response of C_{57}BL/6 male mice to adult *Paragonimus westermani* antigens and sheep red blood cells (SRBC). The mice were immunized with adult *Paragonimus westermani* antigens one day after injection of the present cysteine protease. Group treated with the protease on day-1 (□) and control group (●). Bars represent standard errors. (a) The initial adult *P. wetermani* antigen injection one day after the protease treatment. (b) SRBC injection six months after the initial adult *P. westermani* antigen immunization. (c) adult *P. westermani* antigen injection one year after the initial adult *P. westermani* antigen immunization.

FIG. **5** is a graph showing a footpad response of C_{57}BL female mice to sheep red blood cells (SRBC). The mice were immunized with SRBC one day after injection of various cysteine proteases. Groups treated with plasmin, trypsin, papain, cathepsin B_1 and untreated control (□),collagenase (●), and *P. westermani* cysteine protease (○). Bars represent standard errors.

DETAILED DESCRIPTION OF THE INVENTION

It is an object of the present invention to provide immunosuppressive drugs which, without long continuous administration, suppress both delayed-type hypersensitivity and antibody production against specific antigens and graft tissues and induce immunological tolerance to specific antigens and grafted tissues.

The present invention is characterized by the following description.

(1). The present invention provides an immunosuppressive drug in which cysteine protease comprises its composition.

(2). The present invention provides the immunosuppressive drug of (1) in which the cysteine protease contains a larger amount of acidic amino acids than basic amino acids.

(3). The present invention provides the immunosuppressive drug of (1) or (2) in which the cysteine protease is active in neutral hydrogen ion concentration.

(4). The present invention provides the immunosuppressive drug of (1), (2), or (3) in which the cysteine protease is extracted from the infected larvae of parasitic helminths.

(5). The present invention provides the immunosuppressive drug of (1), (2), (3), or (4) in which the cysteine protease comprises an amino acid sequence described in SEQ ID No. 1.

(6). The present invention provides a cysteine protease having an amino acid sequence described in SEQ ID No. 1.

(7). The present invention provides a cysteine protease gene encoding a polypeptide having an amino acid sequence described in SEQ ID No. 1.

(8). The present invention provides a cysteine protease gene having a base sequence described in SEQ ID No. 2.

The term, "cysteine protease" of the present invention is intended to mean an endopeptidase comprising cysteine residues at amino acid positions, 22, 25, 56, 63, 96, 154, and 202, and a histidine residue at amino acid position 161 (the position is numbered from N terminus in the amino acid

sequence) and having an active center at cysteine and histidine residues.

The immunosuppressive drug of the invention comprises cysteine protease directly obtained by extracting the cell and tissue of organisms including invertebrates such as parasitic helminths to vertebrates such as various animals including mammal, or by cultured cells isolated from organisms described above, or by genetic engineering using recombinant DNA containing a cysteine protease gene (recombinant DNA technique), or by chemical synthesis.

When the cysteine protease of the present invention (hereinafter referred to as "present cysteine protease") is injected within a few days and/or several days before an injection of an antigen, the injection of the present cysteine protease suppresses cell-mediated immune response and/or humoral immune response, which is antibody production, against the antigen, for a long period of time. In addition, such a prior injection of the present cysteine protease allow hosts to take a graft for a long period of time and induce the hosts to acquire immunological tolerance to the graft.

Although the detailed mechanism of how cysteine protease works on the immune system is not clearly understood, it is believed that the immunosuppressive drug of the invention administered to a mammalian host is involved with the antigen presentation of immunocytes, receptors or suppressor T cells, thereby suppressing both delayed-type hypersensitivity and antibody production and inducing the immunological tolerance of the mammalian host.

The present invention will be further described in Examples, which are not intended to limit the scope of the invention.

EXAMPLE

1. Extraction and Purification of Cysteine Protease from Parasitic Helminth

The cysteine protease of the invention was obtained by extracting *Paragonimus westermani* metacercaria with 50 mM imidazole-HCl/pH7.0, subjecting the extract to centrifugation (at 105,000 g, for 60 minutes), loading the supernatant onto Arginine-Sepharose CL-4B affinity chromatography, filtering the eluate on an Ultrogel AcA-54 gel and purifying the filtrate by DEAE-toyopearl S column chromatography. N-terminal 25 amino acids of the purified native cysteine protease is shown in SEQ ID No. 5.

2. Amino Acid and Base Sequence of Present Cysteine Protease

The purified present cysteine protease was emulsified with Freund's complete adjuvant. The emulsion was intravenously injected in the ears of rabbits for immunization, and antiserum against the present cysteine protease was obtained.

About 2×10^4 *Paragonimus westermani* metacercariae were used to obtain mRNA. Total RNA (about 100 μg) was extracted from the pooled material by phenol extraction and poly A^+RNA (about 2 μg) was recovered by passing the total RNA through an oligo (dT)-cellulose column. The mRNA thus obtained was used to construct a cDNA library using the λgt11 vector. The cDNA library was screened by the antiserum and 25 independent positive clones containing about 300–600 bp long insert were obtained. Of 25 clones, two clones having the longest cDNA sequence were designated as λPW6 and λPW24. The two clones were subcloned using pUC118, and the two subclones were named pPW6 and pPW24, respectively. Then, the base sequences of the two clones were determined by the dideoxy nucleotide sequencing method using a T7 sequencing kit (United States Biochemicals, U.S.A.).

E. coli MV1184 was transformed with the subclone pPW6 and the transformant, *E. coli* MV1184/pPW6, was deposited with Fermentation Research Institute, Agency of Industrial Science and Technology and was assigned the accession number FERM BP-3937.

The base sequence and deduced amino acid sequence of pPW6 and pPW24 are shown in SEQ ID Nos. 3 and 4, respectively. Primers were synthesized based on the sequence near the ends of the cDNA insert of the clone and were used to screen the cDNA library again by the PCR.

A base sequence including the entire coding region of the gene of one mature cysteine protease of *Paragonimus westermani* metacercaria was determined (SEQ ID No. 2). The mRNA sequence contains an open reading frame with a significant length. The deduced amino acid sequence is shown in SEQ ID No. 1. The base sequence of the other several cDNA clones strongly suggested that each protease has a few different amino acids and belongs to a closely related protein family. The mature cysteine protease of the invention was found to contain 215 amino acids.

3. Characterization of Amino Acid Sequence of Present Cysteine Protease

The comparison of the primary structures of the present cysteine protease and the cysteine protease of other species (hereinafter referred to as "similar cysteine proteases") has revealed the following features: an amino acid sequence (boxed region) in FIG. 1 is homologous in both the present cysteine protease and similar cysteine proteases; cysteine residues are found at amino acid positions, 22, 25, 56, 63, 96, 154, and 202, and a histidine residue is found at amino acid position 161, the position that is numbered from N terminus in the amino acid sequence. This primary structure suggests that the present cysteine protease has a typical feature of cysteine protease. However, the underlined amino acid sequence of the present cysteine protease is different from those of all the other similar cysteine proteases.

In addition, the base sequences of other clones have revealed some substitutions of amino acid residues: in the present cysteine protease, there are substitutions, Ala→Pro (amino acid position 15), Ser→Glu (21), Argo→Met (58), Val→Ala (59), Gln→Glu (61), Ala→Ser (69), and Tyr→Asp (77), the position that is numbered from N terminus in the amino acid sequence. This amino acid substitution strongly suggests that the primary structure of the present cysteine protease is at least 90% homologous to the other cysteine protease and that the present cysteine protease has a few different amino acids from other cysteine proteases to form its own family. These findings also coincide with variability found in the serine proteases of nematodes and are believed to be the common feature of parasitic helminths.

Furthermore, there is a difference in the number of amino acid residues between cysteine at amino acid position 56 and the following cysteine: 6 amino acid residues are found in the present cysteine protease, papain, chymopapain and papaya proteinase while 8 amino acid residues are found in cathepsin L and H. Based on the known three-dementional structure of papain, it is believed that the present cysteine protease forms a disulfide bridge at amino acid positions 22–63, 56–96 and 154–202, and its active site residue is cysteine at amino acid position 25 and histidine at 161.

The present cysteine protease is composed of more acidic amino acids(14.89–14.96 mole % acidic amino acid such as glutamic acid and aspartic acid) than basic amino acid (10.17–10.23 mole % basic amino acid such as lysine and arginine). The amino acid composition is similar to that of cathepsin L, but different from those of papain, chymopapain and papaya proteinase, which contains more basic amino acids (13.66–14.29 mole %) than acidic amino acids (7.08–8.37 mole %) (see Table 1). In order that a cysteine

protease works well in the neutral or faint acidic environment of the organism, it appears reasonable that the cysteine protease have a high acidic amino acid content. The high acidic amino acid content of the present cysteine protease is believed to be the reason that the present cysteine protease alone has an immunosuppressive effect while other similar cysteine proteases such as papain and the like do not.

The present cysteine protease contains leucine in a large amount (8.10–8.14 mole %) and asparagine and tyrosine in a small amount –3.86 and 5.95–6.58 mole %, respectively), a prominent feature that distinguishes the present cysteine protease from other similar cysteine proteases (Table 1).

TABLE 1

Amino acid composition of a cysteine protease and other homologous cysteine proteases

	Mol %					
Amino acid	CP*	PPA*	CPPA*	PP*	CL*	CH*
Glutamic acid	9.09	5.93	3.73	5.44	9.30	5.23
Leucine	8.10	5.29	5.71	4.85	5.17	5.13
Valine	5.96	7.72	6.80	9.09	6.63	6.25
Glycine	6.00	7.70	7.35	8.04	6.63	6.14
Aspartic acid	5.80	2.44	3.35	2.46	6.12	4.26
Alanine	5.83	4.57	4.52	4.61	5.04	6.02
Lysine	6.37	5.36	11.13	7.56	6.72	7.27
Glutamine	5.31	5.35	5.83	6.48	4.13	5.19
Threonine	4.76	3.05	6.05	3.96	3.37	4.23
Tryptophan	5.19	3.74	2.96	3.02	3.61	3.63
Asparagine	3.36	5.81	5.27	6.83	5.14	5.64
Serine	5.35	5.01	6.48	6.99	7.06	4.86
Cysteine	3.52	3.11	3.51	2.69	3.00	3.45
Methionine	2.17	0.00	0.54	0.00	3.16	3.71
Tyrosine	6.58	12.61	9.86	8.70	8.33	9.65

TABLE 1-continued

Amino acid composition of a cysteine protease and other homologous cysteine proteases

	Mol %					
Amino acid	CP*	PPA*	CPPA*	PP*	CL*	CH*
Arginine	3.80	8.30	3.16	6.44	3.69	1.86
Proline	3.35	4.22	4.59	3.40	3.66	4.91
Phenylalanine	3.00	2.42	3.59	1.83	4.67	5.87
Isoleucine	4.77	6.25	3.80	5.33	2.78	5.60
Histidine	1.69	1.14	1.68	2.29	1.64	1.10

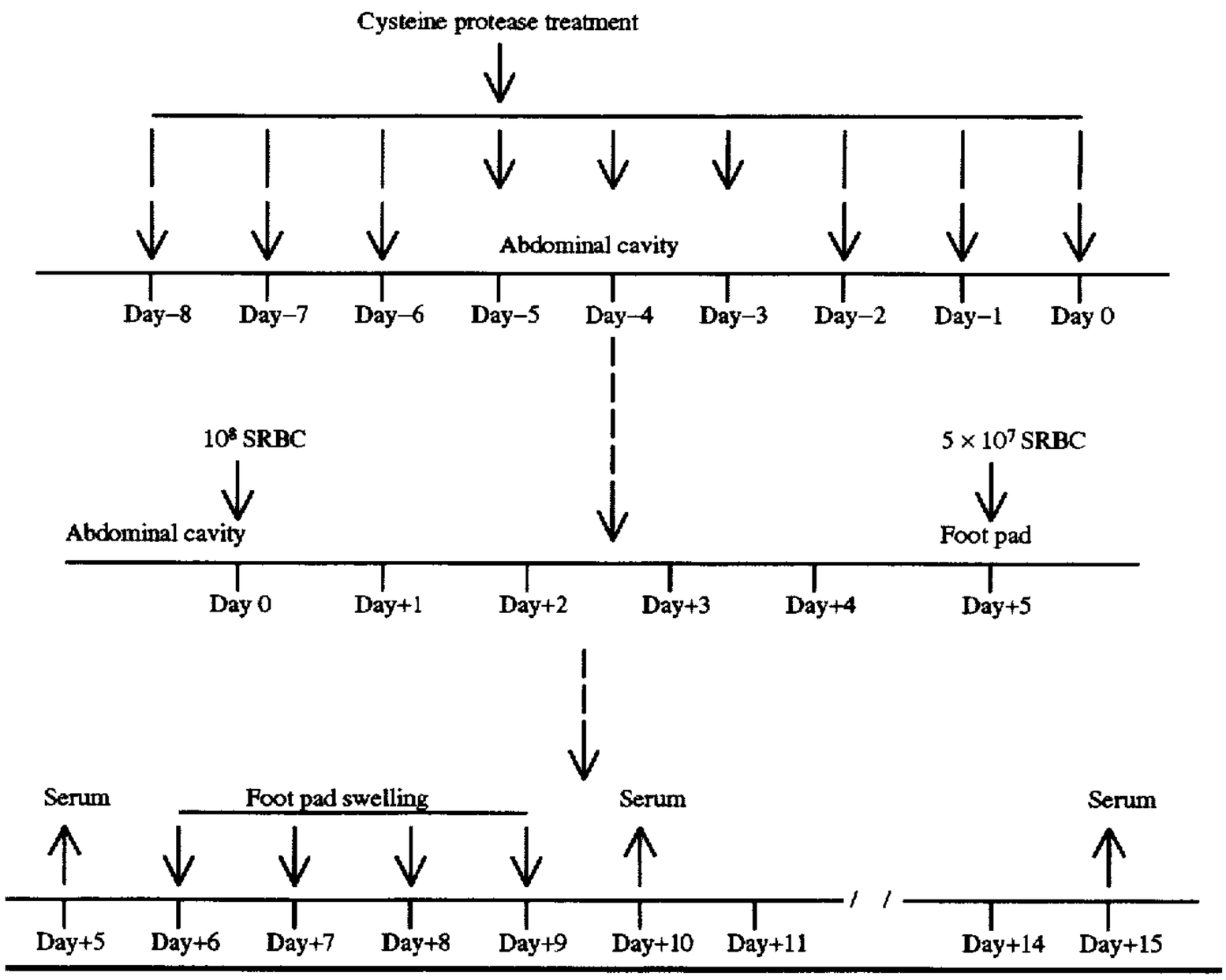
*CP: Cysteine protease, PPA: Papain, CPPA: Chymopapain, PP: Papaya proteinase, CL: Cathepsin L, CH: Cathepsin H

4 Suppressive Effects of Present Cysteine Protease on Cell-mediated Immune Response and Humoral Immune Response.

12-week old C57BL/6 female mice were divided into 10 groups, each group having 6 mice. The present cysteine protease was intraperitoneally administered (100 ng protein per mouse which is an enough concentration to suppress footpad reaction) to the mice, except for a control group, 1, 2, 3, 4, 5, 7, and 8 days before the administration of antigens and on the same day when antigens were administered. The mice were immunized with the intraperitoneal injection of 1×10^8 sheep red blood cells (SRBC) suspended in 0.1 ml of phosphate buffed saline (PBS) as an initial antigen. Five days later, 5×10^7 SRBCs suspended in 0.05 ml PBS as booster was injected to the footpad. Then, degree of swelling (thickness) was measured (Experimental schedule 1 shown in Table 2).

TABLE 2

Experimental schedule 1

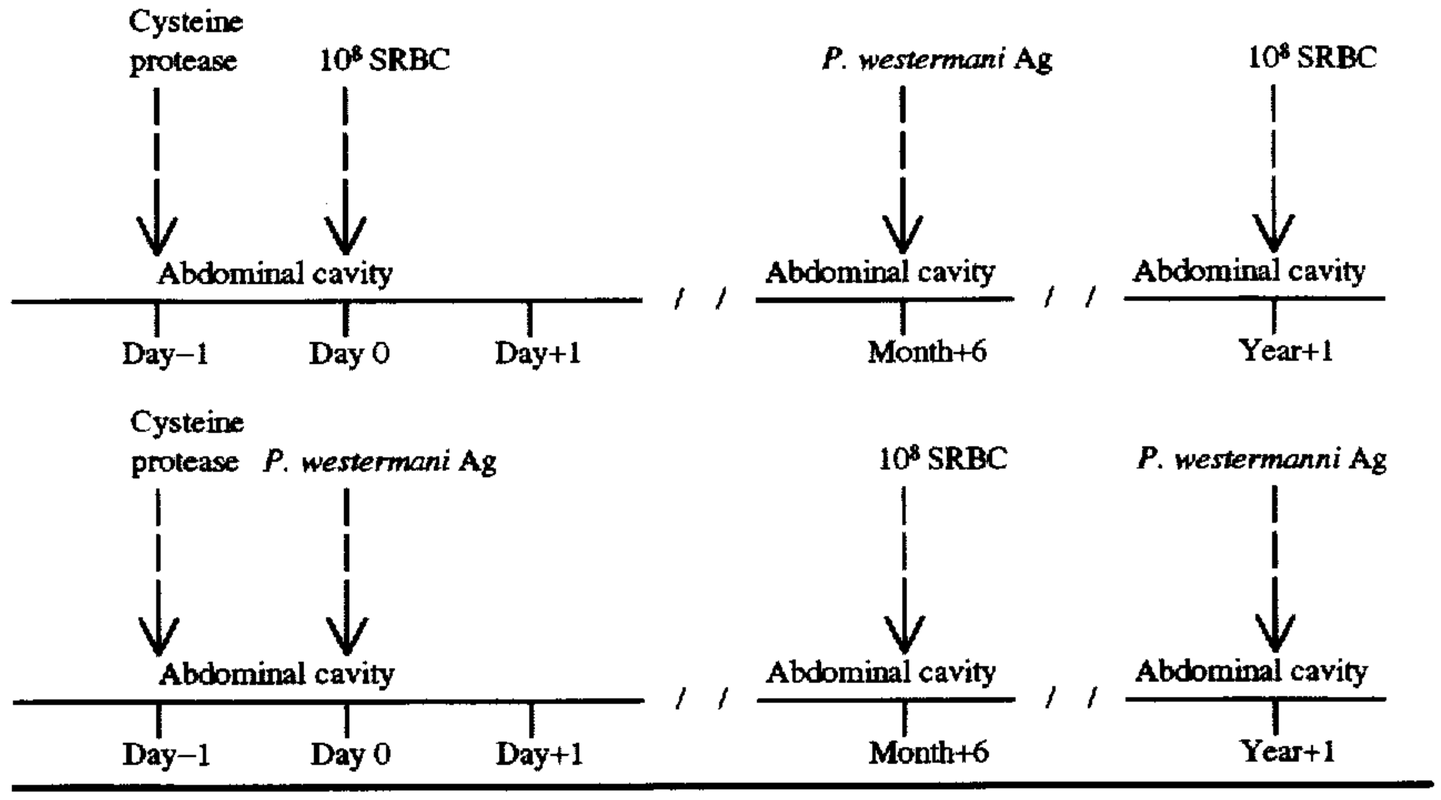


Compared with control, footpad reaction (swelling) was significantly suppressed in the group ($P<0.01$) to which present cysteine protease was administered one day before immunization. This result suggests that administration of the present cysteine prolease suppresses delayed-type hypersensitivity, against SRBC (FIG. 2).

groups, the immunosuppression was specifically occurred to the antigen that was initially injected before one year while no immunosuppression of a footpad reaction was occurred to other antigens (FIGS. 3 and 4).

TABLE A

Experimental schedule 2



Each group of mice was tested for the antibody titer (HA) of blood serum using SRBC. Compared with control, antibody production was significantly suppressed in the group of mice to which the present cysteine protease was administered 4–5 days before the injection of the antigen (Table 3).

TABLE 3

Antibody production in serum of $C_{57}BL/6$ female mice injected intraperitoneally with SRBC immunization at various days after treatment of cysteine protease

Day of CP treatment	Anti-SRBC (HA) titer (Log_2) on 15 days after immunization Mean ± SE
Day 0	6.0 ± 0.26
Day-1	5.5 ± 0.22
Day-2	5.0 ± 0.31
Day-3	4.2 ± 0.31
Day-4	3.0 ± 0.31
Day-5	2.2 ± 0.17
Day-6	3.3 ± 0.37
Day-7	4.5 ± 0.22
Day-8	5.0 ± 0.30
Control	5.5 ± 0.34

CP (Cysteine protease) and SE (Standard error)

5 Immunological Tolerance Induced by Present Cysteine Protease

The present protease (100 ng protein per mouse) was intraperitoneally administered to 12-week old B57BL/6 female and male mice. One day later, a group was immunized with SRBC or adult *Paragonimus westermani* antigens (Experimental schedule 2 shown in Table 4). The group demonstrated the suppression of a footpad reaction against SRBC or adult *Paragonimus westermani* antigens even one year after the initial immunization (FIGS. 3 and 4). In these

As is shown in Experimental schedule 3 in Table 5, when the skin of 8-week old, AKR female mice was implanted to 10-week old, C3H/He female mice to which the present cysteine protease (total amount, 1.5 μg protein) had been administered one and 4 days before implantation, the administered group took the graft for a significantly longer period of time ($P<0.05–0.01$; mean survival time: 100±36) than control (mean survival time: 18±0.5 days) (Table 6).

TABLE 5

Experimental schedule 3

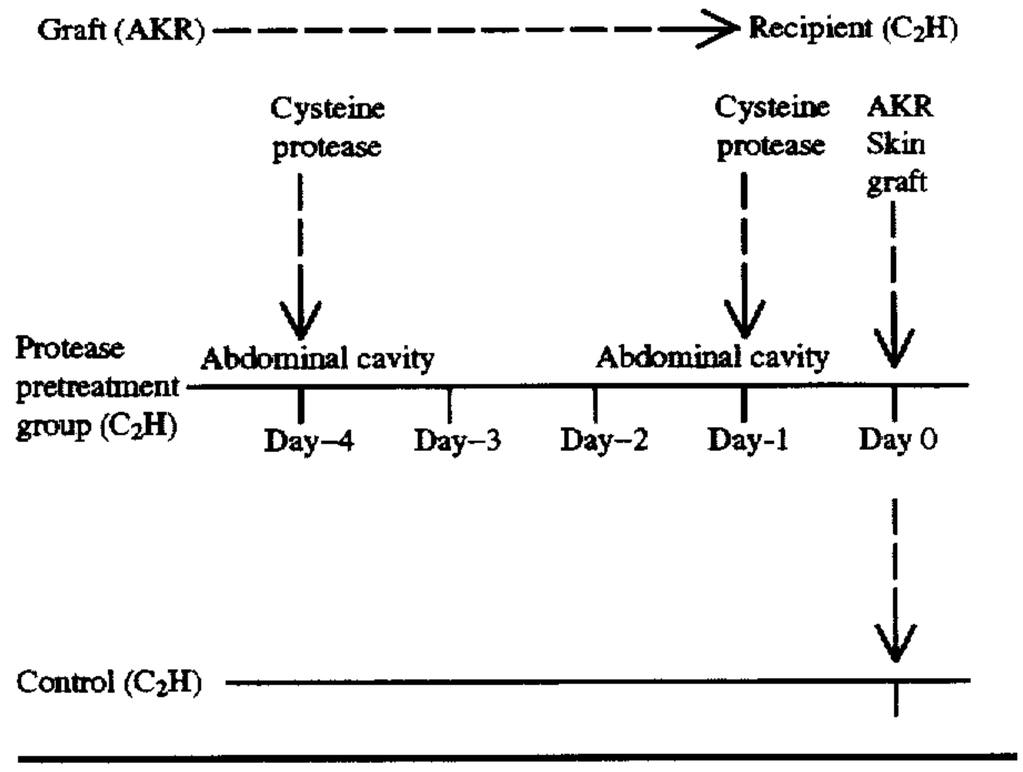


TABLE 6

Effects of CP treatment on the survival of skin allografts

Recipient	Donor of skin grafts	CP dose schedule (μg/Kg) Day-4	CP dose schedule (μg/Kg) Day-1	Skin allograft survival time (days) in individual mice	MST(day) ± SE	P value (t-test)	No. of hair growth	P value (x^2-test)
C_3H/He ♀	ARK ♀	—	—	17,17,18,18,19,20	18 ± 0.5	<0.05	0	<0.01
		30	15	20 > 30 > 60 > 90 > 150 > 250	100 ± 36		5	

The abbreviations are mean survival time (MST), standard error (SE) and cysteine protease (CP)

As is evident from the results, the present cysteine protease intraperitoneally administered to mice was found to induce immunological tolerance to the antigen or the tissue which were injected or implanted immediately after the administration of the present cysteine protease.

6. Immunological Tolerance of Other Protease Species

Most of the different proteases (e.g., 100 ng of Plasmin, Trypsin, Cathepsin B1, Papain, or Collagenase per mouse) intraperitoneally administered to 12-week old, C57BL/6 female mice did not induce immunological tolerance (FIG. 5). Weak suppression of a footpad reaction was observed in the collagenase administered group but no immunosuppression was observed one year later.

As is evident from the above experiment, the administration of the present cysteine protease suppresses delayed-type hypersensitivity and antibody production against specific antigens and implants, and induces immunological tolerance in the mice.

Pharmaceutical agents utilizing the immunosuppressive effects of the present cysteine protease for therapeutic purpose may be in various forms including an injectable solution described in Example, inhalant, ointment, or a lyophilized form. Dosage may depend on how the present cysteine protease is administered and what therapeutic purpose one is in need.

Although the present cysteine protease used in Example is one that is directly extracted from parasitic helminths, an amount of the cysteine protease that is extracted from one parasitic helminth is a very small. For the isolation and purification of the cysteine protease, it not only takes long time but is also costly so that it is desirable for a commercial scale of the preparation of the cysteine protease to produce it in a large amount by cultured animal cells or by genetic engineering using recombinant DNA containing a gene of interest (recombinant DNA technology).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 5

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215
(B) TYPE:amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY:Linear

(i i) MOLECULE TYPE:peptide

(v i) ORIGINAL SOURCE:
(A) ORGANISM:Paragonimus westermani metacecaria
(B) CELL TYPE:
(C) CELL LINE:

(i x) FEATURE:
(A) LOCATION: 5
(B) NAME/KEY: Xaa:Met or Ile

(i x) FEATURE:
(A) LOCATION:15
(B) NAME/KEY:Xaa:Ala or Pro

(i x) FEATURE:
(A) LOCATION:21
(B) NAME/KEY:Xaa:Ser or Glu

(i x) FEATURE:
(A) LOCATION:58
(B) NAME/KEY:Xaa:Arg or Met

(i x) FEATURE:

-continued

(A) LOCATION:59
(B) NAME/KEY:Xaa:Val or Ala

(i x) FEATURE:
(A) LOCATION:61
(B) NAME/KEY:Xaa:Gln or Glu

(i x) FEATURE:
(A) LOCATION:69
(B) NAME/KEY:Xaa:Ala or Ser

(i x) FEATURE:
(A) LOCATION:77
(B) NAME/KEY:Xaa:Tyr or Asp

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:1:

```
Ala Pro Glu Arg Xaa Asp Trp Arg Ala Lys
                5                  10

Gly Ala Val Thr Xaa Val Glu Asn Gln Gly
               15                  20

Xaa Cys Gly Ser Cys Trp Ala Phe Ser Thr
               25                  30

Ala Gly Asn Val Glu Gly Gln Trp Phe Ile
               35                  40

Lys Thr Gly Gln Leu Val Ser Leu Ser Lys
               45                  50

Gln Gln Leu Val Asp Cys Asp Xaa Xaa Ala
               55                  60

Xaa Gly Cys Asn Gly Gly Trp Pro Xaa Ser
               65                  70

Ser Tyr Leu Glu Ile Met Xaa Met Gly Gly
               75                  80

Leu Glu Ser Glu Ser Asp Tyr Pro Tyr Val
               85                  90

Gly Val Glu Gln Thr Cys Ala Leu Asn Lys
               95                 100

Glu Lys Leu Val Ala Lys Ile Asp Asp Ser
              105                 110

Ile Val Leu Gly Pro Glu Glu Glu Asp His
              115                 120

Ala Ala Tyr Leu Ala Glu His Gly Pro Leu
              125                 130

Ser Thr Leu Leu Asn Ala Val Ala Leu Gln
              135                 140

Tyr Tyr Gln Ser Gly Val Leu Lys Pro Thr
              145                 150

Phe Glu Glu Cys Pro Asp Thr Glu Leu Asn
              155                 160

His Ala Val Leu Thr Val Gly Tyr Asp Lys
              165                 170

Glu Gly Asp Met Pro Tyr Trp Ile Ile Lys
              175                 180

Asn Ser Trp Gly Thr Asp Trp Gly Glu Lys
              185                 190

Gly Tyr Phe Arg Leu Phe Arg Gly Asp Cys
              195                 200

Thr Cys Gly Ile Asn Arg Met Ala Thr Ser
              205                 210
```

-continued

Ala Ile Ile Lys Lys
215

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 648
(B) TYPE:nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE:cDNA to mRNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM:Paragonimus westermani metacecaria
(B) CELL TYPE:
(C) CELL LINE:

(i x) FEATURE:

(i x) FEATURE:
(A) LOCATION:15
(B) NAME/KEY:N:T or G

(i x) FEATURE:
(A) LOCATION:43
(B) NAME/KEY:N:G or C

(i x) FEATURE:
(A) LOCATION:48
(B) NAME/KEY:N:T or G

(i x) FEATURE:
(A) LOCATION:61
(B) NAME/KEY:N:T or G

(i x) FEATURE:
(A) LOCATION:62
(B) NAME/KEY:N:C or A

(i x) FEATURE:
(A) LOCATION:66
(B) NAME/KEY:N:C or T

(i x) FEATURE:
(A) LOCATION:123
(B) NAME/KEY:N:A or G

(i x) FEATURE:
(A) LOCATION:173
(B) NAME/KEY:N:G or T

(i x) FEATURE:
(A) LOCATION:176
(B) NAME/KEY:N:T or C

(i x) FEATURE:
(A) LOCATION:181
(B) NAME/KEY:N:C or G

(i x) FEATURE:
(A) LOCATION:205
(B) NAME/KEY:N:G or T

(i x) FEATURE:
(A) LOCATION:213
(B) NAME/KEY:N:C or A

(i x) FEATURE:
(A) LOCATION:229
(B) NAME/KEY:N:T or G

(i x) FEATURE:
(A) LOCATION:237
(B) NAME/KEY:N:C or T

(i x) FEATURE:
(A) LOCATION:306
(B) NAME/KEY:N:G or A

-continued

(i x) FEATURE:
(A) LOCATION:366
(B) NAME/KEY:N:C or T

(i x) FEATURE:
(A) LOCATION:370
(B) NAME/KEY:N:T or C

(i x) FEATURE:
(A) LOCATION:375
(B) NAME/KEY:N:A or T

(i x) FEATURE:
(A) LOCATION:471
(B) NAME/KEY:N:T or C

(i x) FEATURE:
(A) LOCATION:489
(B) NAME/KEY:N:T or G

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:2:

```
GCT CCC GAA CGT ATN GAC TGG CGG GCT AAG          30
GGC GCT GTG ACA NCG GTN GAA AAT CAA GGC          60
NNG TGN GGT TCG TGT TGG GCG TTC TCG ACT          90
GCC GGA AAC GTT GAA GGT CAA TGG TTC ATC         120
AAN ACC GGT CAG CTT GTC AGT CTG AGC AAA         150
CAG CAA TTG GTC GAC TGT GAC ANG GNG GCC         180
NAG GGA TGC AAT GGT GGA TGG CCA NCC AGT         210
TCN TAC CTG GAA ATC ATG NAT ATG GGN GGT         240
TTG GAG TCC GAA AGC GAC TAT CCC TAT GTT         270
GGT GTG GAA CAA ACG TGT GCC CTG AAC AAG         300
GAG AAN CTG GTA GCC AAA ATC GAT GAT TCG         330
ATT GTT CTG GGT CCG GAG GAG GAG GAC CAC         360
GCC GCN TAT NTG GCN GAA CAC GGA CCG TTG         390
AGT ACG CTG CTC AAT GCC GTC GCT CTT CAG         420
TAC TAC CAG TCC GGA GTA CTC AAA CCG ACC         450
TTT GAG GAG TGT CCG GAT ACN GAG TTG AAC         480
CAC GCG GTN CTC ACC GTC GGC TAT GAC AAG         510
GAA GGC GAT ATG CCA TAC TGG ATC ATC AAG         540
AAT AGT TGG GGT ACC GAC TGG GGC GAG AAA         570
GGC TAC TTC CGA CTC TTC CGA GGA GAT TGC         600
ACG TGT GGA ATC AAC CGC ATG GCA ACA TCC         630
GCG ATC ATC AAG AAA TGA                         648
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 648
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE:cDNA to mRNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM:Paragonimus westermani metacecaria

-continued

(B) CELL TYPE:
(C) CELL LINE:

(i x) FEATURE:

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:3:

```
GCT CCC GAA CGT ATT GAC TGG CGG GCT AAG                                  30
Ala Pro Glu Arg Ile Asp Trp Arg Ala Lys
                5                  10

GGC GCT GTG ACA GCG GTT GAA AAT CAA GGC                                  60
Gly Ala Val Thr Ala Val Glu Asn Gln Gly
            15                  20

TCG TGC GGT TCG TGT TGG GCG TTC TCG ACT                                  90
Ser Cys Gly Ser Cys Trp Ala Phe Ser Thr
        25                  30

GCC GGA AAC GTT GAA GGT CAA TGG TTC ATC                                 120
Ala Gly Asn Val Glu Gly Gln Trp Phe Ile
    35                  40

AAA ACC GGT CAG CTT GTC AGT CTG AGC AAA                                 150
Lys Thr Gly Gln Leu Val Ser Leu Ser Lys
45                  50

CAG CAA TTG GTC GAC TGT GAC AGG GTG GCC                                 180
Gln Gln Leu Val Asp Cys Asp Arg Val Ala
            55                  60

CAG GGA TGC AAT GGT GGA TGG CCA GCC AGT                                 210
Gln Gly Cys Asn Gly Gly Trp Pro Ala Ser
        65                  70

TCC TAC CTG GAA ATC ATG TAT ATG GGC GGT                                 240
Ser Tyr Leu Glu Ile Met Tyr Met Gly Gly
    75                  80

TTG GAG TCC GAA AGC GAC TAT CCC TAT GTT                                 270
Leu Glu Ser Glu Ser Asp Tyr Pro Tyr Val
85                  90

GGT GTG GAA CAA ACG TGT GCC CTG AAC AAG                                 300
Gly Val Glu Gln Thr Cys Ala Leu Asn Lys
            95                  100

GAG AAG CTG GTA GCC AAA ATC GAT GAT TCG                                 330
Glu Lys Leu Val Ala Lys Ile Asp Asp Ser
        105                 110

ATT GTT CTG GGT CCG GAG GAG GAG GAC CAC                                 360
Ile Val Leu Gly Pro Glu Glu Glu Asp His
    115                 120

GCC GCC TAT TTG GCA GAA CAC GGA CCG TTG                                 390
Ala Ala Tyr Leu Ala Glu His Gly Pro Leu
125                 130

AGT ACG CTG CTC AAT GCC GTC GCT CTT CAG                                 420
Ser Thr Leu Leu Asn Ala Val Ala Leu Gln
            135                 140

TAC TAC CAG TCC GGA GTA CTC AAA CCG ACC                                 450
Tyr Tyr Gln Ser Gly Val Leu Lys Pro Thr
        145                 150

TTT GAG GAG TGT CCG GAT ACT GAG TTG AAC                                 480
Phe Glu Glu Cys Pro Asp Thr Glu Leu Asn
    155                 160

CAC GCG GTT CTC ACC GTC GGC TAT GAC AAG                                 510
His Ala Val Leu Thr Val Gly Tyr Asp Lys
165                 170

GAA GGC GAT ATG CCA TAC TGG ATC ATC AAG                                 540
Glu Gly Asp Met Pro Tyr Trp Ile Ile Lys
            175                 180

AAT AGT TGG GGT ACC GAC TGG GGC GAG AAA                                 570
```

-continued

```
Asn Ser Trp Gly Thr Asp Trp Gly Glu Lys
                185                 190

GGC TAC TTC CGA CTC TTC CGA GGA GAT TGC                                 600
Gly Tyr Phe Arg Leu Phe Arg Gly Asp Cys
                195                 200

ACG TGT GGA ATC AAC CGC ATG GCA ACA TCC                                 630
Thr Cys Gly Ile Asn Arg Met Ala Thr Ser
                205                 210

GCG ATC ATC AAG AAA TGA                                                 648
Ala Ile Ile Lys Lys ***
                215
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 648
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear

(i i) MOLECULE TYPE:cDNA to mRNA

(v i) ORIGINAL SOURCE:
(A) ORGANISM:Paragonimus westermani metacecaria
(B) CELL TYPE:
(C) CELL LINE:

(i x) FEATURE:

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:4:

```
GCT CCC GAA CGT ATG GAC TGG CGG GCT AAG                                  30
Ala Pro Glu Arg Met Asp Trp Arg Ala Lys
                5                   10

GGC GCT GTG ACA CCG GTG GAA AAT CAA GGC                                  60
Gly Ala Val Thr Pro Val Glu Asn Gln Gly
                15                  20

GAG TGT GGT TCG TGT TGG GCG TTC TCG ACT                                  90
Glu Cys Gly Ser Cys Trp Ala Phe Ser Thr
                25                  30

GCC GGA AAC GTT GAA GGT CAA TGG TTC ATC                                 120
Ala Gly Asn Val Glu Gly Gln Trp Phe Ile
                35                  40

AAG ACC GGT CAG CTT GTC AGT CTG AGC AAA                                 150
Lys Thr Gly Gln Leu Val Ser Leu Ser Lys
                45                  50

CAG CAA TTG GTC GAC TGT GAC ATG GCG GCC                                 180
Gln Gln Leu Val Asp Cys Asp Met Ala Ala
                55                  60

GAG GGA TGC AAT GGT GGA TGG CCA TCC AGT                                 210
Glu Gly Cys Asn Gly Gly Trp Pro Ser Ser
                65                  70

TCA TAC CTG GAA ATC ATG GAT ATG GGT GGT                                 240
Ser Tyr Leu Glu Ile Met Asp Met Gly Gly
                75                  80

TTG GAG TCC GAA AGC GAC TAT CCC TAT GTT                                 270
Leu Glu Ser Glu Ser Asp Tyr Pro Tyr Val
                85                  90

GGT GTG GAA CAA ACG TGT GCC CTG AAC AAG                                 300
Gly Val Glu Gln Thr Cys Ala Leu Asn Lys
                95                  100

GAG AAA CTG GTA GCC AAA ATC GAT GAT TCG                                 330
Glu Lys Leu Val Ala Lys Ile Asp Asp Ser
                105                 110

ATT GTT CTG GGT CCG GAG GAG GAG GAC CAC                                 360
```

-continued

```
Ile Val Leu Gly Pro Glu Glu Glu Asp His
                115                 120

GCC GCT TAT CTG GCT GAA CAC GGA CCG TTG                          390
Ala Ala Tyr Leu Ala Glu His Gly Pro Leu
                125                 130

AGT ACG CTG CTC AAT GCC GTC GCT CTT CAG                          420
Ser Thr Leu Leu Asn Ala Val Ala Leu Gln
                135                 140

TAC TAC CAG TCC GGA GTA CTC AAA CCG ACC                          450
Tyr Tyr Gln Ser Gly Val Leu Lys Pro Thr
                145                 150

TTT GAG GAG TGT CCG GAT ACC GAG TTG AAC                          480
Phe Glu Glu Cys Pro Asp Thr Glu Leu Asn
                155                 160

CAC GCG GTG CTC ACC GTC GGC TAT GAC AAG                          510
His Ala Val Leu Thr Val Gly Tyr Asp Lys
                165                 170

GAA GGC GAT ATG CCA TAC TGG ATC ATC AAG                          540
Glu Gly Asp Met Pro Tyr Trp Ile Ile Lys
                175                 180

AAT AGT TGG GGT ACC GAC TGG GGC GAG AAA                          570
Asn Ser Trp Gly Thr Asp Trp Gly Glu Lys
                185                 190

GGC TAC TTC CGA CTC TTC CGA GGA GAT TGC                          600
Gly Tyr Phe Arg Leu Phe Arg Gly Asp Cys
                195                 200

ACG TGT GGA ATC AAC CGC ATG GCA ACA TCC                          630
Thr Cys Gly Ile Asn Arg Met Ala Thr Ser
        205                 210

GCG ATC ATC AAG AAA TGA                                          648
Ala Ile Ile Lys Lys ***
                215
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE:amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY:linear

(i i) MOLECULE TYPE:peptide

(v i) ORIGINAL SOURCE:
(A) ORGANISM:Paragonimus westermani metacecaria
(B) CELL TYPE:
(C) CELL LINE:

(i x) FEATURE:
(A) OTHER INFORMATION:
The 22nd and 25th residues, Xaa, are to be cysteine which could not be detected with this PTH analyser system. The amino acid sequence shown below is N-terminal 25 amino acids of the purified native cysteine protease.

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```
Ala Pro Glu Ser Ile Asp Trp Arg Glu Lys
                5                  10

Gly Ala Val Ala Pro Val Glu Asp Gln Gly
                15                 20

Ser Xaa Gly Ser Xaa
                25
```

What is claimed is:

1. A composition comprising a biologically active cysteine protease derived from parasitic helminths wherein the amino acid sequence is at least 90% identical to the amino acid sequence of SEQ ID No. 1.

2. The composition of claim 1 wherein the cysteine protease has an amino acid sequence in which the total number of acidic amino acid residues in the sequence exceeds the total number of basic amino acid residues in the sequence.

3. The composition of claim 1 wherein the cysteine protease is active at about pH 7.

4. The composition of claim 2 wherein the cysteine protease is active at about pH 7.

5. The composition of claim 2 wherein the cysteine protease has the amino acid sequence of SEQ ID No. 1.

6. The composition of claim 3 wherein the cysteine protease has the amino acid sequence of SEQ ID No. 1.

7. The composition of claim 4 wherein the cysteine protease has the amino acid sequence of SEQ ID No. 1.

8. A composition comprising a biologically active cysteine protease derived from parasitic helminths wherein the amino acid sequence is at least 90% identical to the amino acid sequence of SEQ ID No. 1 and wherein the amino acid sequence of the cysteine protease has a proline residue at position 15, a glutamate residue at positions 21 and 61, a cysteine residue at positions 22, 25, 56, 63, 96, 154 and 202, a methionine residue at position 58, an alanine residue at position 59, a serine residue at position 69, an aspartate residue at position 77 and a histidine residue at position 161, wherein the amino acid residue positions are relative to the N terminus.

9. The composition of claim 8 wherein the cysteine protease has an amino acid sequence in which the total number of acidic amino acid residues in the sequence exceeds the total number of basic amino acid residues in the sequence.

10. The composition of claim 8 wherein the cysteine protease is active at about pH 7.

11. The composition of claim 8 wherein the cysteine protease is from infected larvae of parasitic helminths.

12. A composition comprising a cysteine protease with an amino acid sequence of SEQ ID number 1.

13. A composition comprising a biologically active cysteine protease derived from parasitic helminths wherein the amino acid sequence is at least 90% identical to the amino acid sequence of SEQ ID No. 1 and wherein the amino acid sequence of the cysteine protease has a proline residue at position 15, a glutamate residue at positions 21 and 61, a cysteine residue at positions 22, 25, 56, 63, 96, 154 and 202, a methionine residue at position 58, an alanine residue at position 59, a serine residue at position 69, an aspartate residue at position 77 and a histidine residue at position 161, wherein the amino acid residue positions are relative to the N terminus wherein the cyateine protease has an amino acid sequence in which the total number of acidic amino acid residues in the sequence exceeds the total number of basic amino acid residues in the sequence wherein the cysteine protease is active at about pH 7.

* * * * *